

THE RELATION OF SOROCARP SIZE TO PHOTOTAXIS IN THE CELLULAR SLIME MOLD, *DICTYOSTELIUM PURPUREUM*¹

JOHN TYLER BONNER AND FRANCES ELIZABETH WHITFIELD

Department of Biology, Princeton University, Princeton, New Jersey

One of the most striking features of the cellular slime molds is orientation towards light during the migrating and culminating phases. This has been known since the work of the early workers, and more recently we were able to show that the sensitivity of this reaction is truly remarkable, although we suggested incorrectly that the light and heat response might be explained by a similar mechanism (Bonner, Clarke, Neely and Slifkin, 1950). Gamble (1953) was the first to show that the light response was so sensitive that it was necessary to postulate a separate photoreceptor and this has been confirmed in detail by Francis (1964), who was even able to obtain a rough action spectrum of the photosensitive pigment.

For some years in our laboratory we have attempted to demonstrate phototaxis in vegetative or aggregation amoebae with no success. Samuel (1961), in particular, paid attention to this problem as has Francis (1964), and we have repeated a number of the obvious experiments during the course of this study. But in no case was any photo orientation demonstrated as it was in Davenport's (1897) early experiments on large soil amoebae.

The purpose of this study was to determine whether or not small cell masses, which would be intermediate in size between single cells and the usual large pseudoplasmodia, would respond to unidirectional light with the same effectiveness as the larger masses, or whether they would show intermediate responses. As will be seen, for a given light intensity, the effectiveness of the response definitely decreases with size. The phototactic response is correlated with sorocarp size.

METHODS

The majority of these experiments were done on *Dictyostelium purpureum* (strain No. 2), although a few comparative studies were made with *Polysphondylium violaceum* (strain No. 6), *P. pallidum* (strain No. 4), *Acytostelium leptosomum*, and *Protostelium mycophaga*. In all cases they were grown on *Escherichia coli* which had been painted on the surface of 2% non-nutrient agar.

When aggregation was largely completed and well formed centers were evident, these were punched out with a small glass tube and placed on the surface of a large block of agar within a lucite box 3 × 3 × 9 cm. in size (Fig. 1). These were in turn put in a darkroom so that each individual center resided at the same given distance from the light source. There was no temperature regulation of the dark

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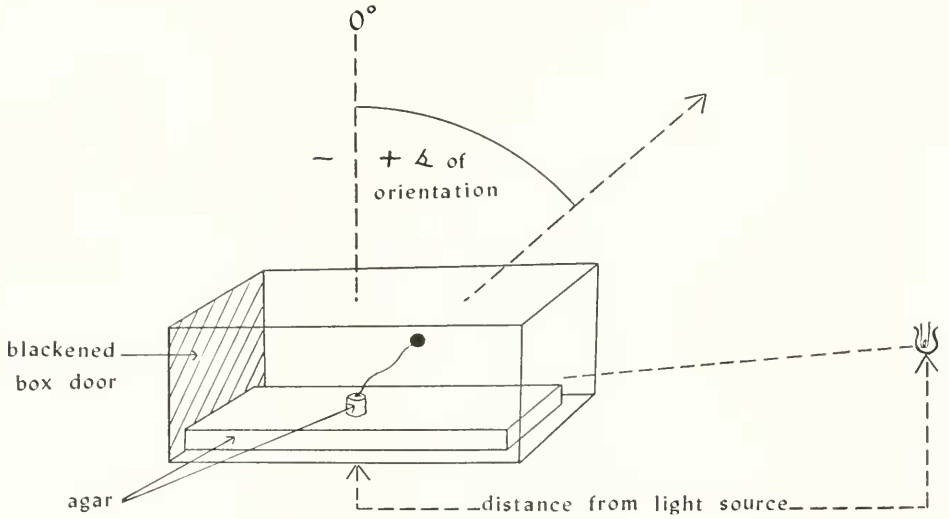


FIGURE I.

room and while the temperature was relatively steady during any one experiment, the temperature at which the experiments were run varied from 24° C. to 27° C.

The light source was a 6.5-volt G.E. headlight lamp No. 1493. It was set with a transformer so that it received 1.14 amps. and 0.90 volts. The intensity of the light was checked before and after an experiment with a photocell and a galvanometer.

After the pseudoplasmodia had fruited, the plastic boxes were placed upon their sides under a dissecting microscope and camera lucida drawings were made of the sorocarps. The angles were determined by drawing a line from the base through the upper end of the stalk at the point where it enters the sorus (Fig. 1). The vertical position was considered 0°, and any deviation towards light was given a positive angle value, while orientation away from the light was given a negative value.

TABLE I

Mean angles of deviation from the perpendicular for different size sorocarps at three different distances from a light source. The standard deviations are given for each mean and the number of cases in parentheses (These are plotted in Figure 2)

Height of sorocarp in mm. (grouped in size classes of ±0.2 mm.)	Angle of deviation from the perpendicular with the light at different distances		
	1 foot	6 feet	18 feet
0.2	10° ± 23°(9)	3° ± 20°(7)	1° ± 19°(10)
0.6	10° ± 30°(14)	-4° ± 10°(8)	7° ± 28°(12)
1.0	42° ± 26°(14)	3° ± 14°(14)	-3° ± 14°(16)
1.4	37° ± 31°(13)	21° ± 28°(12)	2° ± 22°(22)
1.8	48° ± 36°(10)	33° ± 17°(9)	-4° ± 14°(22)
2.2	53° ± 15°(5)	38° ± 23°(6)	8° ± 20°(20)
2.6	52° ± 18°(6)	35° ± 19°(9)	2° ± 50°(15)

RESULTS

As can be seen from Table I and Figure 2, the experiment was run at three different distances, 1, 6, and 18 feet. Although the standard deviations show considerable variability in the response, it is clear that at one foot the larger sorocarps responded while the smaller ones did not. At 18 feet there was no

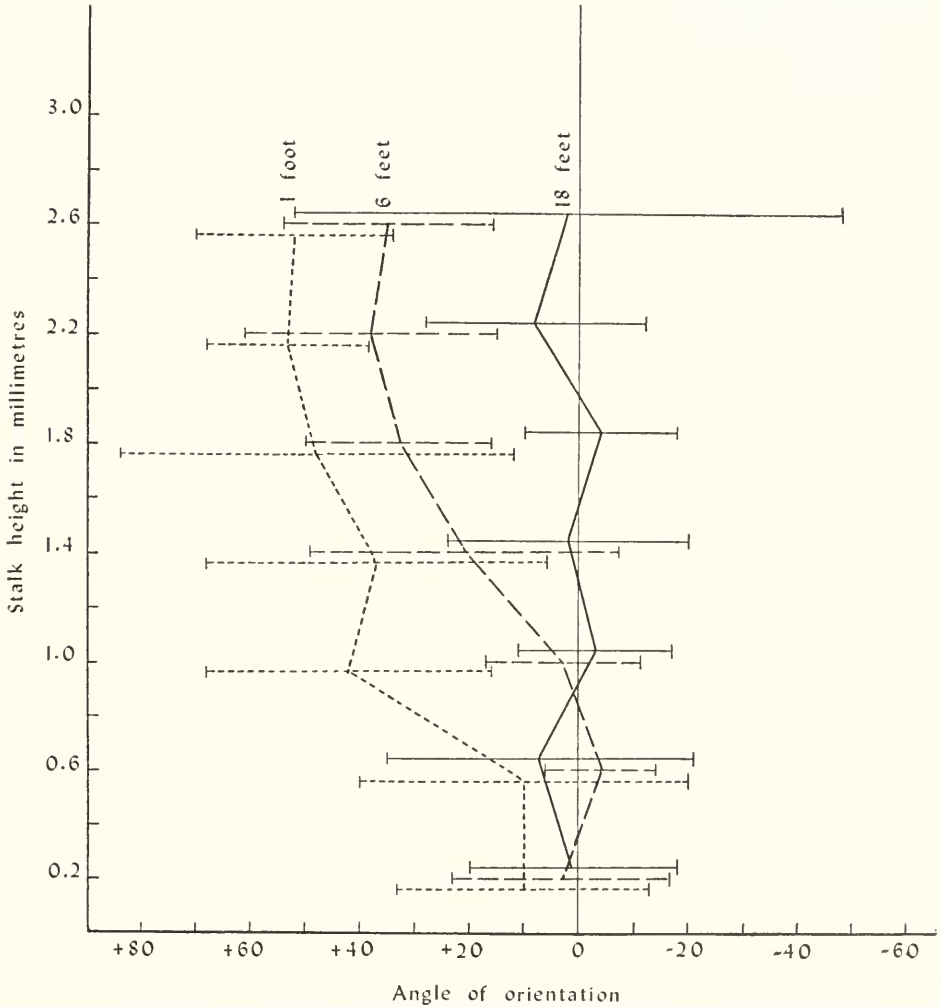


FIGURE 2.

response, even of the largest sorocarp. In fact this agrees with a control run in the total darkness, involving 60 sorocarps. At 6 feet there was an intermediate response. For instance if one looks at the sorocarps that are 1 mm. long, then only those at 1 foot are oriented while those at 6 and 18 feet are not. However, at 1.4-mm. the ones at 6 feet from the light source are definitely oriented, although the

angle of orientation is less than those at one foot. It should be added that this same result was obtained in a preliminary experiment involving 59 cases in which the intensity of the light was changed rather than changing the distance. This experiment, however, has the serious objection that the spectrum of the tungsten filament changes with intensity and therefore the data have not been included here.

These results were repeated in a brief preliminary experiment comparing five species. In *Protostelium*, which has no aggregative phase and consists of a single cell raised on a minute stalk (Olive and Stoianovitch, 1960), and in *Acytostelium*, which is a very small species with an acellular stalk (Raper and Quinlan, 1958), there was no orientation at all, even with strong light intensities. The remaining three were tested at the same light intensity and *Polysphondylium pallidum* (21 cases) was the least sensitive, *Dictyostelium purpureum* (37 cases) next, and *Polysphondylium violaceum* (18 cases) the most sensitive and showed the lowest

TABLE II

Mean angles of deviation from the perpendicular for different-size sorocarps of Polysphondylium pallidum. In the first column of angles two sorocarps are repelling each other, and in the second column a single sorocarp is orienting away from a vertical wall of agar. In both instances the distance between the sorocarp base and the other sorocarp or agar varies from 0 to .53 mm. The number of cases is indicated in parentheses

Height of sorocarp in mm. (Grouped in size classes of $\pm .1$ mm.)	Angle of deviation from perpendicular	
	Two sorocarps	Sorocarp and agar wall
.2	35°(1)	7°(1)
.4	31°(8)	16°(2)
.6	19°(12)	5°(5)
.8	20°(8)	13°(9)
1.0	9°(2)	8°(5)
1.2	20°(1)	17°(3)

threshold. It is of interest that only the main stalk in *Polysphondylium* showed orientation; the small side whorls never leaned towards the light, which might be expected on the basis of their small size.

During the course of these experiments the question arose as to whether light might directly affect the rate of movement within the cell mass. This was tested by subjecting migrating masses of *Dictyostelium discoideum* to alternating periods of light and dark (with the microscope lamp in the darkroom) and measuring the rate of movements. In 18 separate experiments, most of them run for at least four hours, the light was alternately turned on and off at hourly intervals. The overall average rate during the dark periods is almost identical to that during the light period (Light = 1.6 mm./hr.; Dark = 1.7 mm./hr.) and if one scores whether light has produced an increase or decrease in speed (or conversely the dark), both occur with equal frequency. A few further experiments were run with the light and dark alternating at 10-minute intervals and again there was no difference in the rate. These results are consistent with those of Francis (1964), who showed amoebae separated from a slug and subjected to strong increases in light intensity did not exhibit any change in speed.

Besides the effect of light it should be recorded that two other types of orientation were tested.

By putting the plastic boxes on end in the total darkness it was possible to test the effect of gravity. The results do not show, as might be expected, that the large sorocarps droop. In 54 cases there is no clear downward trend, but the variance appears greater than the right-side-up controls in the dark and the right-side-up sorocarps 18 feet distant from light (Fig. 2). It would be interesting to know if the mechanism which controls orientation in the dark has difficulties in operating with precision when the sorocarp arises on a vertical wall.

Presumably the method of orientation in the dark is entirely by gas gradients (Bonner and Dodd, 1962a). The old data from those experiments were re-examined and it is possible to compare the amount of repulsion between sorocarps and the size of the sorocarp. It is obvious from the results shown in Table II that the small sorocarps are as effective in orienting to gas gradients as large ones, and that this is true for a number of species.

DISCUSSION

The discussion of these results will be divided into two sections: the mechanism of orientation, and the question of adaptation.

Unfortunately, these experiments tell us very little about the mechanism of orientation, but then perhaps this is not too surprising because despite all the detailed work on phototropism in *Phycomyces*, it is still far from understood. Orientation in the slime molds is similar to *Phycomyces* in that Francis (1964) showed that a small beam of light hitting one side of slug will cause the slug to bend away from that side, thus showing a parallel to Buder's (1920) experiment in *Phycomyces*. During the course of our work we repeated another experiment of Buder and placed the slime molds in mineral oil. As in *Phycomyces* they oriented away from the light and all of this substantiates Francis' (1964) conclusion that the lens effect is operative in the slime molds.

Unlike *Phycomyces*, there is no evidence that light affects the speed of movement, even for brief periods. From this Francis (1964) makes the reasonable suggestion that perhaps the light might be affecting the extensibility of the slime sheath which in turn directs the movement.

Another difference is that in *Phycomyces* the smaller sporangiophores are more sensitive to a given unilateral light than large ones, as Castle (1964) has shown. This is exactly contrary to the results here. From this we must conclude that the limiting factors are different for the two systems, but what they might be for the slime molds is difficult to surmise. It is not just light intensity, for if the lens effect operates, it should if anything be more effective in the small fruiting bodies at a given light intensity. Therefore, there must also be some limitation within the sorocarp itself. Something within the cell mass is quantitatively below threshold, but the threshold can be raised to some extent if the unidirectional light intensity is raised.

It is interesting that this should be in marked contrast to the orienting effects of gas gradients. Here the smallest sorocarps are as responsive as the large ones. The fact that single amoebae can also orient very effectively in chemical gradients,

such as the acrasin gradient, food gradients (Samuel, 1961) and mutual repulsion gradients (Samuel, 1961), is perhaps consistent with the notion that there is no size threshold for these chemical effects.

To turn now to the question of adaptation, if orientation to light has adaptive value, then clearly there will be a selection pressure for large slime molds. Increase in size, however, may result in other features which are inadapative, and some sort of balance must be reached.

Size is to some degree fixed for a particular species or a particular strain and this is in part due to the spacing mechanism (Bonner and Dodd, 1962b; Bonner and Hoffman, 1963) and in part due to another mechanism which operates at higher amoeba densities (Hohl and Raper, 1964). Since there are different-sized species in nature we must presume that there are a number of adaptive advantages and disadvantages in size. This same question has been examined for different-sized *Hydra* by Slobodkin (1964), where he considers the whole question in terms of strategy, balancing the advantages and disadvantages in different ways. Here we can say that the ability to orient towards light improves with increased size, and if for any one species, in a particular environment, this is advantageous, there will be selection for size increase.

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SUMMARY

Large fruiting bodies of the cellular slime mold, *Dictyostelium purpurcum*, orient more effectively towards a source of light of low intensity than do small ones. The threshold of sensitivity can be changed either by changes in size of the sorocarp or by changes in the light intensity. However, in chemical gradients small cell masses are as sensitive as large ones. Therefore, if orientation to light is of adaptive value, selection pressure for size increase would be expected.

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